

WHAT IS CLAIMED IS:

1. A method of detecting or quantifying a target nucleic acid having a predetermined sequence in a specimen comprising:

5 (a) preparing a probe A and a probe B,  
said probe A being a first probe which has a sequence F' complementary to a first partial sequence F of the target nucleic acid and a binding molecule bound to the sequence F', and

10 said probe B being a second probe which has a sequence S' complementary to a second partial sequence S of the target nucleic acid and a flag bound to the sequence S', where said flag is a double-stranded sequence and has a marker substance in one of the  
15 double strand;

(b) hybridizing the first probe A with the first partial sequence F of the target nucleic acid and hybridizing the second probe B with the second partial sequence S of the target nucleic acid;

20 (c) ligating the first probe A and the second probe B both being hybridized with the target nucleic acid, thereby obtaining a probe (A+B);

(d) binding the binding molecule to a substance capable of being paired up therewith, thereby  
25 recovering the probe (A+B); and

(e) recovering a single-stranded nucleic acid having the marker substance of the double stranded

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nucleic acid constituting the flag and detecting or quantifying the marker substance, thereby detecting or quantifying the target nucleic acid in the specimen.

2. A method of detecting or quantifying target  
5 nucleic acids N1-Nn (n is an integer of 2 or more),  
each having a predetermined sequence, in a specimen,  
comprising:

(a) preparing probes A1-An (n is an integer of 2  
10 or more) and probes B1-Bn (n is an integer of 2 or  
more),

said probes A1-An being first probes which  
respectively have sequences F1'-Fn' (n is an integer of  
2 or more) complementary to first partial sequences  
F1-Fn (n is an integer of 2 or more) of the target  
15 nucleic acids and a binding molecule bound to each of  
the sequences F1'-Fn', and

said probes B1-Bn (n is an integer of 2 or more)  
being second probes which respectively have sequences  
S1'-Sn' (n is an integer of 2 or more) complementary to  
20 second partial sequences S1-Sn (n is an integer of 2 or  
more) of the target nucleic acids and flags bound to  
the sequences S1'-Sn', where each of said flags is a  
double-stranded sequence and has a marker substance in  
one of the double strand; and

25 (b) respectively hybridizing the first probes  
A1-An with the first partial sequences F1-Fn of the  
target nucleic acids, and simultaneously hybridizing

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the second probes B1-Bn with the second partial sequences S1-Sn of the target nucleic acids, respectively;

5 (c) respectively ligating the first probes A1-An and the second probes B1-Bn, both being hybridized with the target nucleic acids, respectively, thereby obtaining probes (A1+B1)-(An+Bn) (n is an integer of 2 or more);

10 (d) binding the binding molecule to a substance capable of being paired up therewith, thereby recovering the probes (A1+B1)-(An+Bn); and

15 (e) recovering a single-stranded nucleic acid having the marker substance from the double-stranded nucleic acid constituting each of the flags and detecting or quantifying the marker substance, thereby detecting or quantifying each of the target nucleic acids N1-Nn in the specimen.

20 3. A method of detecting or quantifying a target nucleic acid having a predetermined sequence, in a specimen, comprising:

25 (a) preparing a probe A and a probe B, said probe A being a first probe which has a sequence F' complementary to a first partial sequence F of the target nucleic acid and a tag sequence Tg bound to the sequence F', and

said probe B being a second probe which has a sequence S' complementary to a second partial sequence

S of the target nucleic acid and a marker substance bound to the sequence S'

(b) mixing the probe A, the probe B, and the specimen, thereby hybridizing the probe A with the first partial sequence F of the target nucleic acid and simultaneously hybridizing the probe B with the second partial sequence S of the target nucleic acid;

(c) ligating the probe A and the probe B, both being hybridized with the target nucleic acid, thereby obtaining a probe (A+B);

(d) dissociating the probe (A+B) from the target nucleic acid;

(e) hybridizing the tag sequence Tg with a sequence Tg' complementary to the tag sequence Tg, thereby recovering the probe (A+B); and

(f) detecting or quantifying the marker substance in the probe (A+B) recovered, thereby detecting or quantifying the target nucleic acid in the specimen.

4. A method of detecting or quantifying nucleic acids N1-Nn, each having a predetermined sequence, in a specimen, comprising:

(a) preparing probes A1-An (n is an integer of 2 or more) and probes B1-Bn (n is an integer of 2 or more),

said probes A1-An being first probes which respectively have sequences F1'-Fn' (n is an integer of 2 or more) complementary to first partial sequences

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5           said probes B1-Bn being second probes which  
respectively have sequences S1'-Sn' (n is an integer of  
2 or more) complementary to second partial sequences  
S1-Sn (n is an integer of 2 or more) of the target  
nucleic acids N1-Nn and the marker substance bound to  
10 each of the sequences S1'-Sn' of the target nucleic  
acid;

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(e) hybridizing sequences Tgl-Tgn respectively with sequences Tgl'-Tgn' complementary to the tag sequences Tgl-Tgn, thereby recovering the probes

(A1+B1)-(An+Bn); and

(f) detecting or quantifying the marker substance  
in the probes (A1+B1)-(An+Bn) recovered, thereby  
detecting or quantifying the target nucleic acids N1-Nn  
in the specimen.

5           5. A method of detecting or quantifying a target  
nucleic acid having a predetermined sequence in a  
specimen, comprising:

(a) preparing a probe A and a probe B,

10           said probe A being a first probe which has a  
sequence F' complementary to a first partial sequence F  
of the target nucleic acid and a tag sequence Tg bound  
to the sequence F', and

15           said probe B being a second probe which has a  
sequence S' complementary to a second partial sequence  
S of the target nucleic acid, a flag sequence FL bound  
to the sequence S', and a marker substance bound to the  
flag sequence FL;

20           (b) mixing the probe A, the probe B, and the  
specimen, thereby hybridizing the probe A with the  
first partial sequence F of the target nucleic acid and  
simultaneously hybridizing the probe B with the second  
partial sequence S of the target nucleic acid;

25           (c) ligating the probe A and the probe B, both  
being hybridized with the target nucleic acid, thereby  
obtaining a probe (A+B);

(d) dissociating the probe (A+B) from the target

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nucleic acid;

(e) hybridizing the tag sequence Tg contained in the probe (A+B) with a sequence Tg' complementary to the tag sequence Tg, thereby dissociating the probe (A+B); and

(f) recovering a portion containing at least the probe B from the probe (A+B) hybridized with the sequence Tg';

(g) hybridizing the flag sequence FL recovered with a nucleic acid sequence FL' complementary to the flag sequence FL, thereby specifically recovering the portion containing at least probe B; and

(h) selectively detecting the marker substance contained in the portion containing at least the probe B recovered, thereby detecting or quantifying the target nucleic acid in the specimen.

6. A method of detecting or quantifying nucleic acids N1-Nn (n is an integer of 2 or more), each having a predetermined sequence, in a specimen, comprising:

(a) preparing probes A1-An (n is an integer of 2 or more) and probes B1-Bn (n is an integer of 2 or more),

said probes A1-An being first probes which respectively have sequences F1'-Fn' (n is an integer of 2 or more) complementary to first partial sequences F1-Fn of the target nucleic acids N1-Nn (n is an integer of 2 or more), respectively, and tag sequences

Tg1-Tgn bound to the sequences F1'-Fn', respectively,  
and

said probes B1-Bn being second probes which  
respectively have sequences S1'-Sn' (n is an integer of  
2 or more) complementary to second partial sequences  
S1-Sn (n is an integer of 2 or more) of the target  
nucleic acids N1-Nn, flag sequences FL1-FLn bound to  
the sequences S1'-Sn', and a marker substance bound to  
each of the flag sequences FL1'-FLn';

(b) mixing the probes A1-An, the probes B1-Bn,  
and the specimen, hybridizing probes A1-An respectively  
with the first partial sequences F1-Fn of the target  
nucleic acids N1-Nn, and simultaneously hybridizing the  
probes B1-Bn with the second partial sequences S1-Sn of  
the target nucleic acids N1-Nn, respectively;

(c) respectively ligating probes A1-An and second  
probes B1-Bn, both being hybridized with the target  
nucleic acids, thereby obtaining probes (A1+B1)-(An+Bn)  
(n is an integer of 2 or more);

(d) dissociating the probes (A1+B1)-(An+Bn) from  
the target nucleic acids;

(e) hybridizing tag sequences Tg1-Tgn contained  
in the probes (A1+B1)-(An+Bn) with sequences Tg1'-Tgn'  
complementary to the tag sequences Tg1-Tgn, thereby  
dissociating the probes (A1+B1)-(An+Bn); and

(f) recovering portions respectively containing  
at least the probes B1-Bn, from the probes



(A1+B1)-(An+Bn) hybridized with the sequence Tg1'-Tgn';

(g) hybridizing the flag sequences FL1-FLn with nucleic acid sequences FL1'-FLn' complementary to the flag sequences FL1-FLn, thereby specifically recovering the portions respectively containing at least probes B1-Bn; and

(h) selectively detecting the marker substance contained in the portions respectively containing at least the probes B1-Bn recovered, thereby detecting or quantifying the target nucleic acids N1-Nn in the specimen.

7. A method of detecting or quantifying nucleic acids N1-Nn (n is an integer of 2 or more), each having a predetermined sequence, in a specimen, comprising:

(a) preparing probes A1-An (n is an integer of 2 or more) and probes B1-Bn (n is an integer of 2 or more),

said probes A1-An being first probes which respectively have sequences F1'-Fn' (n is an integer of 2 or more) complementary to first partial sequences F1-Fn of the target nucleic acids N1-Nn (n is an integer of 2 or more) and tag sequences Tg1-Tgn bound to the sequences F1'-Fn', respectively, and

said probes B1-Bn being second probes which respectively have sequences S1'-Sn' (n is an integer of 2 or more) complementary to second partial sequences S1-Sn (n is an integer of 2 or more) of the target

nucleic acids N1-Nn, and a marker substance bound to each of the sequences S1'-Sn'

(b) mixing the probes A1-An, the probes B1-Bn, and the specimen, thereby hybridizing probes A1-An respectively with the first partial sequences F1-Fn of the target nucleic acids N1-Nn, and simultaneously hybridizing the probes B1-Bn with the second partial sequences S1-Sn of the target nucleic acids N1-Nn, respectively;

(c) respectively ligating probes A1-An and second probes B1-Bn, both being hybridized with the target nucleic acids N1-Nn, thereby obtaining probes (A1+B1)-(An+Bn) (n is an integer of 2 or more);

(d) hybridizing tag sequences Tg1-Tgn with sequences Tg1'-Tgn' complementary to the tag sequences Tg1-Tgn, thereby recovering the probes (A1+B1)-(An+Bn); and

(e) detecting or quantifying the marker substance contained in the probes (A1+B1)-(An+Bn) recovered, thereby detecting or quantifying the target nucleic acids N1-Nn in the specimen,

wherein Tm values of the tag sequences Tg1-Tgn are higher than Tm values of sequences F1-Fn and sequences S1-Sn.

8. A method of detecting or quantifying a target nucleic acid having a predetermined sequence in a specimen comprising:

(a) preparing a probe A and a probe B,  
said probe A being a first probe which has a  
sequence F' complementary to a first partial sequence F  
of the target nucleic acid and a binding molecule bound  
5 to the sequence F', and

said probe B being a second probe which has a  
sequence S' complementary to a second partial sequence  
S of the target nucleic acid and a flag sequence FL  
consisting of 4 units bound to the sequence S', where  
10 said flag FL sequence hybridizes with a sequence FL'  
bound to the sequence S' to form a double-stranded  
sequence; and

(b) mixing the probe A, probe B and the specimen,  
thereby hybridizing the probe A with the first partial  
15 sequence F of the target nucleic acid, and  
simultaneously hybridizing the second probe B with the  
second partial sequence S of the target nucleic acid;

(c) ligating the probe A and the probe B, both  
being hybridized with the target nucleic acid, thereby  
20 obtaining a probe (A+B);

(d) binding the binding molecule to a substance  
capable of being paired up therewith, thereby  
recovering the probe (A+B); and

(e) denaturing the double-stranded flag sequence  
25 of the probes (A+B) recovered into single-stranded flag  
sequence;

(f) hybridizing the single-stranded flag sequence

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with two primers one of which has a binding molecule B  
and the other of which has a marker substance L, and  
extending the primers to form a complementary strand of  
the flag sequence FL, thereby obtaining a double  
5 strand;

(g) binding a binding molecule B with a substance  
capable of being paired with the binding molecule B,  
thereby recovering the double strand; and

(h) detecting or quantifying the target substance  
10 L, thereby detecting or quantifying the target nucleic  
acid in the specimen.

9. A method of detecting or quantifying target  
nucleic acids N1-Nn (n is an integer of 2 or more),  
each having a predetermined sequence, in a specimen,  
15 comprising:

(a) preparing probes A1-An (n is an integer of 2  
or more) and probes B1-Bn (n is an integer of 2 or  
more),

said probes A1-An being first probes which  
20 respectively have sequences F1'-Fn' (n is an integer of  
2 or more) complementary to first partial sequences  
F1-Fn (n is an integer of 2 or more) of the target  
nucleic acids and a binding molecule bound to each of  
the sequences F1'-Fn', and

25 said probes B1-Bn (n is an integer of 2 or more)  
being second probes which respectively have sequences  
S1'-Sn' (n is an integer of 2 or more) complementary to

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second partial sequences S1-Sn (n is an integer of 2 or more) of the target nucleic acids, and flag sequences FL1-FLn each consisting of 4 units, bound to the sequences S1'-Sn', where said flag sequences FL1-FLn hybridize respectively with sequences FL1'-FLn' bound to the sequences S1'-Sn' to form double-stranded sequences; and

(b) mixing the probes A1-An, the probes B1-Bn, and the specimen, thereby hybridizing probes A1-An respectively with the first partial sequences F1-Fn of the target nucleic acids N1-Nn, and simultaneously hybridizing the probes B1-Bn with the second partial sequences S1-Sn of the target nucleic acids N1-Nn;

(c) respectively ligating the probes A1-An and the probes B1-Bn, both being hybridized with the target nucleic acids N1-Nn, thereby obtaining probes (A1+B1)-(An+Bn);

(d) binding each of the binding molecules to a substance capable of being paired up therewith, thereby recovering the probes (A1+B1)-(An+Bn); and

(e) denaturing double-stranded flag sequences of the probes (A+B)-(An+Bn) recovered into single-stranded flag sequences;

(f) hybridizing the single-stranded flag sequences FL1-FLn with two primers one of which has a binding molecule B and the other of which has a marker substance L, and extending the two primers, to form

complementary strands of the flag sequences FL1-FLn,  
thereby obtaining double strands;

(g) binding a binding molecule B with a substance  
capable of being paired therewith, thereby recovering  
5 the double strands; and

(h) detecting or quantifying the marker substance  
L, thereby detecting or quantifying the target nucleic  
acids N1-Nn in the specimen.

10 10. A method of detecting or quantifying a target  
nucleic acid having a predetermined sequence in a  
specimen comprising:

(a) preparing a probe A and a probe B,  
said probe A being a first probe which has a  
sequence F' complementary to a first partial sequence F  
15 of the target nucleic acid and a binding molecule bound  
to the sequence F', and

said probe B being a second probe which has a  
sequence S' complementary to a second partial sequence  
S of the target nucleic acid and a flag consisting of 4  
20 units bound to the sequence S', where said flag FL is a  
double-stranded sequence; and

(b) mixing the probe A, the probe B, and the  
specimen, thereby hybridizing the probe A with the  
first partial sequence F of the target nucleic acid and  
25 simultaneously hybridizing the probe B with the second  
partial sequence S of the target nucleic acid;

(c) ligating the probe A and the probe B, both

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being hybridized with the target nucleic acid, thereby obtaining a probe (A+B);

(d) binding the binding molecule to a substance capable of being paired up therewith, thereby recovering the probe (A+B); and

(e) denaturing the double-stranded nucleic acid constituting the flag into single-stranded nucleic acid;

(f) amplifying the single-stranded nucleic acid present in a liquid phase by PCR, thereby performing an encode reaction;

(g) performing transcription of a sequence FL' complementary to the single stranded flag sequence obtained by the encode reaction, by use of two primers one of which is a primer having another binding molecule and the other of which is a primer having a marker substance, thereby performing a decode reaction;

(h) binding said another binding molecule to a substance being paired up therewith, recovering a nucleic acid molecule obtained by the decode reaction; and

(i) detecting or quantifying the marker substance, thereby detecting or quantifying the target nucleic acid.

11. A method of detecting or quantifying target nucleic acids N1-Nn (n is an integer of 2 or more), each having a predetermined sequence, in a specimen,

comprising:

(a) preparing probes A1-An (n is an integer of 2 or more) and probes B1-Bn (n is an integer of 2 or more),

5           said probes A1-An being first probes which respectively have sequences F1'-Fn' (n is an integer of 2 or more) complementary to first partial sequences F1-Fn (n is an integer of 2 or more) of the target nucleic acids and a binding molecule bound to each of the  
10           sequences F1'-Fn', and

              said probes B1-Bn (n is an integer of 2 or more) being second probes which respectively have sequences S1'-Sn' (n is an integer of 2 or more) complementary to second partial sequences S1-Sn (n is an integer of 2 or  
15           more) of the target nucleic acids and flag sequences FL1-FLn each consisting of 4 units, bound to the sequences S1'-Sn';

              (b) mixing the first probes A1-An, the second probes B1-Bn, and the specimen, thereby hybridizing the  
20           probes A1-An respectively with the first partial sequences F1-Fn of the target nucleic acids N1-Nn and simultaneously hybridizing the probes B1-Bn with the second partial sequences S1-Sn of the target nucleic acids N1-Nn, respectively;

25           (c) respectively ligating the probes A1-An and the probes B1-Bn, both being hybridized with the target nucleic acids N1-Nn, thereby obtaining probes

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(A1+B1)-(An+Bn) (n is an integer of 2 or more);

(d) binding the binding molecule to a substance capable of being paired up therewith, to recover the probes (A1+B1)-(An+Bn), and thereafter performing an  
5 encode reaction of each of the flags FL1-FLn; and

(e) performing a decode reaction of the sequences FL1'-FLn' complementary to the flags FL1-FLn obtained by the encode reaction; and

(f) detecting or quantifying the nucleic acid  
10 molecules obtained by the decode reaction, thereby detecting or quantifying the target nucleic acids N1-Nn in the specimen.

12. A method of detecting or quantifying target nucleic acids N1-Nn (n is an integer of 2 or more),  
15 each having a predetermined sequence, in a specimen, comprising:

(a) preparing probes A1-An (n is an integer of 2 or more) and probes B1-Bn (n is an integer of 2 or more),

20 said probes A1-An being first probes which respectively have sequences F1'-Fn' (n is an integer of 2 or more) complementary to first partial sequences F1-Fn (n is an integer of 2 or more) of the target nucleic acids and a binding molecule bound to each of  
25 the sequences F1'-Fn', and

said probes B1-Bn (n is an integer of 2 or more) being second probes which respectively have sequences

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S1'-Sn' (n is an integer of 2 or more) complementary to second partial sequences S1-Sn (n is an integer of 2 or more) of the target nucleic acids and flag sequences FL1-FLn each consisting of 4 units, bound to the sequences S1'-Sn', respectively,

(b) mixing the probes A1-An, the probes B1-Bn, and the specimen, thereby hybridizing probes A1-An respectively with the first partial sequences F1-Fn of the target nucleic acids N1-Nn, and simultaneously hybridizing the probes B1-Bn with the second partial sequences S1-Sn of the target nucleic acids N1-Nn, respectively;

(c) respectively ligating the probes A1-An and the probes B1-Bn, both being hybridized with the target nucleic acids N1-Nn, thereby obtaining probes (A1+B1)-(An+Bn);

(d) binding each of the binding molecules to a substance capable of being paired up therewith to recover the probes (A1+B1)-(An+Bn), and thereafter performing an encode reaction for each of the flags FL1-FLn; and

(e) performing a decode reaction of the sequences F11'-FLn' complementary to the flags FL1-FLn (n is an integer of 2 or more) obtained by the encode reaction; and

(h) detecting the nucleic acid molecules obtained by the decode reaction, thereby detecting or

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quantifying the target nucleic acids N1-Nn in the specimen,

wherein 2 units of 4 units are sequences functioning as primers for PCR amplification.

5        13. A method of detecting or quantifying a target nucleic acid having a predetermined sequence in a specimen comprising:

(a) preparing a probe A and a probe B,

10        said probe A being a first probe which has a sequence F' complementary to a first partial sequence F of the target nucleic acid and a binding molecule bound to the sequence F', and

15        said probe B being a second probe which has a sequence S' complementary to a second partial sequence S of the target nucleic acid and a flag consisting of 4 units bound to the sequence S', where said flag FL is a double-stranded sequence and said 4 units consist of SD, D0, D1 and ED each having an arbitrary sequence, bounded to each other sequentially in the order  
20        mentioned; and

(b) mixing the probe A, the probe B, and the specimen, thereby hybridizing the probe A with the first partial sequence F of the target nucleic acid and simultaneously hybridizing the probe B with the second  
25        partial sequence S of the target nucleic acid;

(c) ligating the probe A and the probe B both being hybridized with the target nucleic acid, thereby

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obtaining a probe (A+B);

(d) binding the binding molecule to a substance capable of being paired up therewith, thereby recovering the probe (A+B); and

5 (e) denaturing the double-stranded nucleic acid constituting the flag into a single-stranded nucleic acid;

(f) hybridizing the single-stranded nucleic acid obtained in a liquid phase with sequences complementary to sequences D11-D1n labeled with a marker substance, as primers,

(g) extending the primers hybridized

(h) denaturing a double-stranded nucleic acid having primers into a single-stranded nucleic acid;

15 (i) hybridizing the sequences D01-D0n specifically with the primers extended to detect or quantify the marker substances included in the sequences D01-D0n, thereby detecting or quantifying the target nucleic acids.

20 14. The method according to claims 10 to 12, wherein the decode reaction comprises, where said flag(s) FL is a double-stranded sequence and said 4 units consist of SD, D0, D1 and ED each having an arbitrary sequence, bound to each other sequentially in  
25 the order mentioned,

(i) performing PCR for a single-stranded sequence encoded using SD sequence to which a binding molecule

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(ii) binding a binding molecule bound to the SD sequence to a substance capable of being paired up therewith, thereby recovering a PCR product;

5 (iii) denaturing the PCR produce into a single strand

(iv) hybridizing the single strand with primers D11'-D1n' labeled;

(v) extending the primers;

10 (vi) denaturing the primers extended into single strands;

(vii) hybridizing extended single strands of the primers with sequences D01-D0n to detect or quantify marker substances included in that sequences D01-D0n, thereby detecting or quantifying the target nucleic acid.

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15. The method according to claims 10 to 12, wherein the decode reaction comprises, where said flag FL is a double-stranded sequence and said 4 units

20 consist of SD, D0, D1 and ED each having an arbitrary sequence, bound to each other sequentially in the order mentioned; and

(i) performing PCR for a single-stranded sequence encoded using SD sequence to which a binding molecule

25 is attached and ED sequence, as primers;

(ii) binding the binding molecule bound to the SD sequence to a substance capable of being paired up

